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Review

Pesticidal activity of *Tithonia diversifolia* (Hemsl.) A. Gray and *Tephrosia vogelii* (Hook f.); phytochemical isolation and characterization: A review

N. Kerebba [a], A.O. Oyedeji [b], R. Byamukama [c], S.K. Kuria [d], O.O. Oyedeji [a,*]

[a] Department of Chemistry, University of Fort Hare, P/BagX1314, Alice 5700, South Africa
[b] Department of Chemical and Physical Sciences, Walter Sisulu University, P/BagX1, Mthatha 5117, South Africa
[c] Department of Chemistry, Makerere University, P.O. Box 7062, Kampala, Uganda
[d] Department of Biological and Environmental Sciences, Walter Sisulu University, P/BagX1, Mthatha 5117, South Africa

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ABSTRACT

This paper presents a critical review on the current status of knowledge of the chemistry of *Tithonia diversifolia* and *Tephrosia vogelii*. The review explores the isolation, characterization of bioactive compounds and pesticidal evaluation of the extracts and isolates from the two plants. Existing concerns are over efficacy which indicate that most pesticidal studies on *T. diversifolia* have been conducted using plant extracts and a few of their fractions, which does not fully demonstrate compound effectiveness in pest control, and thus little progress in developing new products. Additionally, the variability in the occurrence of pesticidal compounds within a plant material such as that of *T. vogelii* can affect its end-use as botanical pesticides resulting in instances where farmers report no pesticidal activity of the same species. These challenges can result in the low adoption of pesticidal plants by farmers. The gaps in the knowledge of the chemistry of biological activity entail that the chemical basis for the activity of crude extracts needs to be comprehended while considering them as generation plants for pesticidal use on various crops pests. Furthermore discussions have been made on challenges of isolating and characterizing complex crude extracts. Emerging concerns over their toxicity and safety of bioactive compounds and their metabolites to the environment have also been explored. The review further discusses health-related aspects of bio-markers of biological activity from the crude extracts as reported in other researches. This needs to be studied in these plants' pesticidal applications which could trigger a new course of further research.

Contents

* Corresponding author.
E-mail address: ooyedeji@ufh.ac.za (O.O. Oyedeji).

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1. Introduction

Recent reports indicate that extracts of locally available plants can be effective as crop protectants against pre-harvest and post-harvest pests (Khater, 2012; Kareru et al., 2013). However, there is still low adoption for use of these plants by smallhold farmers. Distinct variation in the phytocompounds of pesticidal plants makes these plants less exploited (Sarasan et al., 2011). The efficacy of the crude extract against pests is important but results would be improved if the knowledge of the chemistry of pesticidal activity is accompanied leading to the isolation of pure bioactive components. Bioactivity may be due to a single compound or due to a large number of structurally related and unrelated phytochemicals which contribute to the effect (Heinrich et al., 2005). The effect thus may be additive or synergistic. Additive effects are created when the combined effect is equal to the sum of the effects of the individual components, while synergistic effects are created when the combination of bioactive substances exert effects that are greater than the sum of the effects of the individual components (Schmidt et al., 2008). There is need to characterize the majority if not all, of the compounds in an extract so that the synergistic effect of the mixture is comprehended (Williamson, 2001; Schmidt et al., 2007; Heinrich, 2008). This would lead to a phytochemical fingerprinting of the extract mixture. A phytocomplex consisting of a combination of different substances, both active principles and other plant components can contribute to the overall biological effect (Donno et al., 2015). The aim of this review is to evaluate the chemistry of pesticidal activity of *T. vogelii* and *T. diversifolia*. *Tephrosia vogelii* shows chemical variability within its species which can therefore compromise its efficacy as source of botanical pesticide. Previous research shows that some non-volatile extracts of *T. vogelii* leaf materials are non-pesticidal based on rotenoid absence while others are pesticidal based on rotenoid presence (Stevenson et al., 2012). To date there is no existing report on to whether there are chemical varieties based on the components of the essential oils of this plant, i.e. the extent of chemical variability of the activity reported in the existing literature. Chemical variability may also occur due to variations in the components of the essential oils of plants when grown at different locations (Kamanula et al., 2017). The review further suggests a study of synergistic action of the binary extracts of *T. vogelii* and *T. diversifolia* after former showing better joint pesticidal action with other plants. In addition, a thorough study of the stability of rotenoid compounds; bioactive compounds of *T. vogelii* under storage conditions is important. Lack of specific information about compounds responsible for some pesticidal effects of *T. diversifolia* on many insect pests could be one of the reasons limiting wide adoption for use and thus little progress in developing new products. The existing and emerging concerns over efficacy and safety of the compounds isolated from different plants can also affect those of *T. diversifolia* for pesticide application. We thus critically summarize the current traditional uses of these plants as botanical pesticides, their phytochemistry, safety and stability of active principles. The review further recommends a study related to the safety of pesticide active ingredients and their break down metabolites despite having been proved that they are environmentally benign compared to synthetic pesticides.

Tithonia diversifolia (Fig. 1) known as wild sunflower or tree marigold is a well-known herb used traditionally in developing countries with agricultural benefits due to insecticidal properties (Casta-O-Quintana et al., 2013). It is a flowering plant in the Asteraceae family (Ayeni et al., 1997) and an ornamental medicinal plant native to South and Central America (Carter, 1978; Zhai et al., 2010). In East Africa, it is common in field boundaries, grasslands and disturbed lands in regions of altitudes ranging from 550 to 1950 m above sea level, mean annual temperature of 15–31 °C and mean annual rainfall of 850–2000 mm (Kandungu et al., 2015). In Uganda, it is found in almost all regions of the country especially in disturbed places. Farmers in Uganda use *T. diversifolia* to manage field and storage pests (Mwine et al., 2011). Its use though is not well-adopted for this purpose. It has reportedly been used against field pests and stored pests of tomatoes, beans and brassicas pests making it good for general consideration in southern and eastern parts of African countries such as Malawi, Kenya, Zimbabwe and many more (Stevenson et al., 2010). The plant flowers and produces seeds throughout the year. The seeds are dispersed by wind, water and animals enhancing its invasive nature (Ayeni et al., 1997; Chagas-Paula et al., 2012).

Fig. 1. *T. diversifolia*, Source: *World agroforestry center:* Species database. 2015.

Fig. 2. *T. vogelii*, Source: *World agroforestry center:* Species database. 2015.

Tephrosia vogelii (Fig. 2), belonging to the family Fabaceae, commonly known as fish poison (Neuwinger, 2004) is native to tropical Africa(Matovu and Olila, 2007). It is a soft woody branching herb with dense foliage and can grow up to 0.5–4.0 m tall (Mwaura et al., 2015). It is found in varying habitats such as savanna-like vegetation, grasslands, forest margins and shrubland, wastelands and fallow fields in some parts of Africa (Stevenson et al., 2012). Attempts have been made in eastern and southern Africa to promote *T. vogelii* for wider application as a pesticide source through earlier reports (Kamanula et al., 2011; Nyirenda et al., 2011). It occurs in climates with an annual rainfall of 850–2650 mm, annual mean temperature of 12.5–26.2 °C and is found up to 2100 m above sea level (Mwaura et al., 2015). It is encountered most in cultivated areas in Malawi for traditional pesticide promotion (Stevenson et al., 2010).

2. Method

Literature on published work of *T. diversifolia* and *T. vogelii* was obtained from pesticidal plant databases and publishers such as Google Scholar, Science Direct, Wiley, Royal Society of Chemistry, American Chemical Society, Scopus, SciFinder. The keywords: *Tithonia diversifolia*, *Tephrosia vogelii*, phytochemical, isolation, characterization, chemical variation, insecticidal, antifeedant, repellence, oviposition, chemotype, toxicity were used to source for the data. In addition words like volatile and non-volatile components were made use of during the search.

3. Results and discussion

3.1. Pesticidal activities of T. diversifolia and T. vogelii

3.1.1. Pesticidal activities of T. diversifolia

Tithonia diversifolia is said to be effective on aphids, weevils, and whiteflies when leaves or seeds are used as a cold infusion (Anjarwalla et al., 2016). Mkenda et al. (2015) found that *T. diversifolia* extract is active against aphids and beetles on common bean plants (*Phaseolus vulgaris* L.). Radhakrishnan and Prabhakaran (2014), reported that the aqueous extract of the plant possesses moderate effect on red spider mite *Oligonychus coffeae* Nietner; one of the major pests infesting tea plantations. Ethanolic extracts from *T. diversifolia* flowers together with *Psychotria prunifolia* H.B.K Steyerm (Rubiaceae) leaves caused higher repellency and mortality in *Sitophilus zeamais* Motsch. adults in corn grains than the other three species from the Brazilian Cerrado biome (*Adenocalymma nodosum* L.G. Lohmann (Bignoniaceae) leaves, *Dimorphandra mollis* Benth (Fabaceae) flowers, *Senna obtusifolia* L. (Fabaceae) leaves) (Tavares et al., 2014). In this study *T. diversifolia* flowers extract applied to corn grains showed class III repellency according to the Preference Index for *S. zeamais* and grain weight loss (3.28 ± 0.45%). The powder and ethanol extracts of *T. diversifolia* leaves showed mortality, inhibition of oviposition and adult emergence of cowpea seed bruchid *Callosobruchus maculatus* F. (Coleoptera, Bruchidae) (Adedire and Akinneye, 2004). In a study reported by Adedire and Akinneye (2004), the leaf ethanol extract had a higher bioactivity on oviposition, adult emergence, and mortality of *C. maculatus*. The mortality reached 100% at higher concentrations (3, 4 and 5%) within 24 h of extract application according to this report. This mortality rate could be achieved when 1% and 2% ethanol extract is administered after 48 h of application, the authors suggest. Accordingly the mortality was 73.3 and 93.3% at 1 and 2%, respectively, after 24 h and mortality was 76–98% at 3–5% with the powder of the plant (Adedire and Akinneye, 2004). *Tithonia diversifolia*, via its monoterpenes has been previously reported to be repellent against insects such as *Phoebis sennae Amphitrite* Feisthamel, *Pieris brassicae* L., *Tatochila autodice blanchardi* Butler, *Tatochila mercedis mercedis* Eschscholtz, *Battus polydamas archidamas* Boisduval, *Cosmosatyrus chilensis* Guérin, *Vanessa carye* Hübner, *Helephila venusta* Hayward, *Culex pipiens pallens* Rank and *Castnia psittachus* Molina (Urzua, 2002; Won-Sik et al., 2002) as well as host-seeking nymphs of *Ixodes ricinus* L. (Acari: Ixodidae) (Jaenson et al., 2006; Pålsson et al., 2008; El-Seedi et al., 2012). It has also been shown that the volatile oil and different extracts of *T. diversifolia* from the leaves exhibit repellent property in which the protection period of the volatile oil at different concentrations (10, 50, and 100%) was measured against the bites of *Anopheles gambiae* Ssand, *Aedes aegypti* L., and *Culex quinquefasciatus* Say (Diptera, Culicidae) in human volunteers (Oyewole et al., 2008). The protection time at 10 and 50% volatile oil was an average of 100 and 160 min, respectively (Oyewole et al., 2008). At 100%, a significant difference between the protection times against three mosquito species was noticed, with a higher repellent effect against *A. gambiae* (200 min) making it act as a bioinsecticide (Oyewole et al., 2008). Antifeedant activity was reported by Ambrósio et al. (2008) on *Chlosyne lacinia* Geyer larvae (Lepidoptera) using dichloromethane (CH_2Cl_2) leaf rinse extracts of *T. diversifolia* at concentrations of 1–5% of fresh leaf. According to this study, the larvae avoided discs rinsed with this extract that was rich in sesquiterpene lactones. Therefore, the deterrent effect was attributed to the presence of such chemical constituents. Pavela et al. (2016) evaluated the potential of *T. diversifolia* leaf methanolic extract and its ethyl acetate fraction for acute and chronic toxicity and for oviposition inhibitory effects against two-spotted spider mite *Tetranychus urticae* Koch. This study revealed that the ethyl acetate extract exhibited anti-oviposition against *T. urticae*. In this report, the ethyl acetate extract effective doses at 50% and 90% application (ED_{50} and ED_{90}, respectively) were 44.3 and 121.5 mg/cm^3, respectively. The mortality exceeded 50% in acute toxicity while the chronic toxicity assays of the methanolic extract gave lethal dose 50% (LD_{50}) of 41.3 mg/cm^3 and LD_{90} of 98.7 mg/cm^3 (Pavela et al., 2016). Adoyo et al. (1997) reported that the leaf extracts from *T. diversifolia* have insecticidal activities against termites due to biologically active compounds. In a related study, Oyedokun et al. (2011), tested different concentrations (12.5, 25, 50, 66.7 and 75% *v*/*v*) in solution of water and ethanolic extracts of *T. diversifolia* against harvester termites *Macrotermes bellicosus* Smeathman (Isoptera: Termitidae). The mortality of the aqueous and ethanolic extracts was 42–88% and 36–68%, respectively. Leaf water extract of *T. diversifolia,* repelled and caused mortality of four insect pests of honey bees namely acrobat ant (*Crematogaster lineolata* Say) small hive beetle (*Aethina tumida* Murray), lesser wax moth (*Achroia grisella* Fabricius) and greater wax moth (*Galleria mellonella* L.) (Olufemi et al., 2015). Therefore, the repellent activity of the essential oil of *T. diversifolia* (Oyewole et al., 2008), its insect feeding deterrent activities (Ambrósio et al., 2008), and insecticidal properties (Taiwo and Makinde, 2005) make it a reliable plant species for both laboratory and field pest control and management agent. However specific information about compounds responsible for pesticidal effects of *T. diversifolia* is inadequate since most bioassays are on plants' crude extracts with only one study so far conducted with pure isolated compounds to *C. maculatus*. The applications of crude extract do not fully demonstrate compound effectiveness in pest control, and thus little progress in the development of new products. The phytochemical knowledge of this plant against several insect pests is therefore still much wanting.

3.1.2. Pesticidal activities of T. vogelii

Tephrosia vogelii is a widely used pesticidal plant and as a source of nutrients to the soil (Stevenson et al., 2012). It has largely been used in southern and eastern Africa to control field pests rather than storage pests (Reuben et al., 2006). Previous study indicate that this plant is very effective in controlling a number of hard-to-kill field insects including: cucumber beetle, leafhoppers, squash bugs, flea beetles, harlequin bug, spittlebugs, thrips, scales, and some fruit worms (Reuben et al., 2006). Anjarwalla et al. (2013) reported its efficacy in controlling bruchids in beans and cowpeas.

Some research has confirmed its insecticidal, antifeedant, and repellent effects against golden flea beetle *Aphthona whitfieldi* Bryant (Coleoptera: Chrysomelidae) (Igogo et al., 2011). Dried leaves of *T. vogelii* were found to have the potential of protecting stored legume seeds from damage by the bruchids as used by farmers in southern Africa (Stevenson et al., 2010). Its water extract of oven dried leaves, seed and stem exhibited some molluscicidal activity against *Biomphalaria pfeifferi* Krauss. I. (Kloos et al., 1987). Its leaf water extract has also been used to control larval stages of mosquitoes and is effective against soft-bodied insects and mites including red spider mites with 20% *w*/*v* giving mortality of more than 90% to the pests (McDavid and Lesseps, 1995). Elwell and Maas (1995), observed that *T. vogelii* aqueous extract (warm water) provides the best protection against aphids. When *T. vogelii* was mixed with groundnuts at a ratio of 1:40, in Democratic Republic of Congo, the powder exhibited 98.8% mortality of the groundnut borer, *Caryedon serratus* Hoi. (Coleoptera:Bruchidae) after 13 days (Delobel and Malonga, 1987). *Tephrosia vogelii* acetone leaf extract showed feeding deterrent activity against cabbage butterfly larvae, *Pieris rapae* Boisd (Shin, 1989). In a related experiment, *T. vogelii* mixture with *Piper cubeba* L. (Piperaceae) extract hexane and its individual hexane leaf extract was effective against crucifer pests such as cabbage aphid *Brevicoryne brassicae* L. (Nugroho et al., 2009). Similarly, powdered (sun-dried) leaves was shown to effectively control maize weevils, the larger grain borer, cowpea and bean bruchids in grain storage (Ayoub, 1999). Farmers in Malawi, Zambia and Zimbabwe use *T. vogelii* as a dip to protect cattle from ticks and as a fish poison to catch fish for food, though now many countries term this as illegal (CMMYT, 1991; Kaposhi, 1992; Gadzirayi et al., 2010). Crude extract from leaves of *T. vogelii* is used to control ticks and worms in the Ugandan animal production systems (Matovu and Olila, 2007). *Tephrosia vogelii* leaf water extract was more active than *Petiveria alliacea* L. root water extracts and was ranked equal to the synthetic insecticide Decis in reducing the population density and damage of field pest of cowpeas (Adebayo et al., 2007). This study was aimed at evaluating the effectiveness of botanical pesticides for the control of insect pests from southern guinea savannah of Nigeria in cowpea field *C. maculatus* (Adebayo et al., 2007). Alao et al. (2012) carried out on-farm evaluation of individual *T. vogelii* fresh leaf water extract and *P. alliacea* root water extract and their mixture against *Megalurothrips sjostedti* Trybom and *Apion varium* Wagner, on cowpeas (*Vigna unguiculata* L Walp and found that the two insect pests were effectively controlled by the botanical insecticides compared to the untreated plants. In another study by Mudzingua et al. (2013), three plants; *T. vogelii, Allium sativum* L. *and Solanum incanum* L. were tested against aphids (*Brevicoryne brassicae* L.) in rapeseed (*Brassica napus* L.). *Tephrosia vogelii* extract was the most active among the three plants (Mudzingua et al., 2013). In another study by Nailufar and Prijono (2017), *T. vogelii* leaf and *Piper aduncum* L. (Piperaceae) fruit ethyl acetate joint extract mixtures were more effective than individual *T. vogelii* or *P. aduncum* extracts in controlling second-instar larvae of cabbage head caterpillar, *Crocidolomia pavonana* L. (Lepidoptera:Crambidae). The authors noted that a ratio of 5:1 for *P. aduncum* to *T. vogelii* showed the strongest synergistic effect on *C. pavonana* at both LC50 (0.254) and LC95 (0.256) levels. A binary mixture containing both *T. vogelii* leaf and *P. cubeba* L. (Piperaceae) fruit ethyl acetate extract (5:9, *w*/*w*) was reported to be more toxic against *C. pavonana* larvae than separate extracts of both samples, furthermore, the mixture indicated a synergistic action (Abizar and Prijono, 2010). In a related study, synergistic action from a tertiary mixture of *Brucea javanica* L., *P. aduncum* and *T. vogelii* extract against *C. pavonana* larvae (Lina et al., 2013). However a joint action of binary mixtures of *T. diversifolia* and *T. vogelii* has not been reported for most of these pests the individual plants have shown to be effective against.

3.2. Current phytochemical studies on T. diversifolia and T. vogelii

3.2.1. Phytochemical studies on some pesticidal activities of T. diversifolia

Many phytocompounds have been isolated from *Tithonia* species, including sesquiterpenoids, diterpenoids, flavonoids and chlorogenic acids derivatives (Chagas-Paula et al., 2012). More than 150 compounds of these secondary metabolites have been isolated from *T. diversifolia* (Zhao et al., 2012) including the sesquiterpenoid mostly sesquiterpene lactones for example tirotundin, tagitinin A, tagitinin C, diversifol and many more (Table 1) (Kuo and Chen, 1998; Gu et al., 2002; Ambrósio et al., 2008; Lin, 2012). The sesquiterpene lactones from *T. diversifolia* fall under germacranolides, eudesmanolides and guaianolides groups. The germacranolides constituting the majority of the most studied sesquiterpene lactones. Other sesquiterpenes that are related to artemisinic acids: artemisinic acid analogue, artemisinic acid derivative, artemisinic acid derivative were also isolated (Bordoloi et al., 1996). Pentacyclic diterpenes namely ent-kaurenoic acid has been found in the leaves and its glandular trichomes of *T. diversifolia* (Ambrósio et al., 2008; Pérez et al., 2009). Flavonoids such as luteolin and nepetin were also found in the leaf trichomes (Ambrósio et al., 2008). Furthermore, flavone derivative hispidulin was isolated by Kuroda et al. (2007) and Pereira et al. (1997). Pantoja Pulido et al. (2017) isolated phenolic compounds from the leaf butanolic extract of *T. diversifolia* which exhibited good antioxidant potential. The chemotypes of the essential oils from *T. diversifolia* have also been described by various researchers (Lamaty et al., 1991; Elufioye and Agbedahunsi, 2004; Moronkola et al., 2007). The main group of essential oil compounds is composed of terpenes and terpenoids, and the other of aromatic and aliphatic constituents, all characterized by low molecular weight (Bakkali et al., 2008). Moronkola et al. (2007) found leaf and flower oil to comprise an abundance of α-pinene, β-caryophyllene, germacrene D, β-pinene and 1,8-cineole, β-caryophyllene and bicyclogermacrene. In this study, aliphatic fatty acids and a diterpenoid compound; sandaracopimaradiene, were present in the flower but could not be detected in the leaf oil. The sesquiterpene lactones 1β-methoxydiversifolin, tagitinin A, and tagitinin C were the major products in addition to α-pinene, β-caryophyllene, β-pinene, germacrene D and 1,8-cineole found during the extraction and characterization of essential oils from the flowers of *T. diversifolia* using a gas chromatography coupled to flame ionization detector (Chukwuka and Ojo, 2014). Thus the non-volatile fractions of *T. diversifolia* are a rich source of flavonoids and sesquiterpene lactones, while the essential oil comprises predominantly monoterpene hydrocarbons (Moronkola et al., 2007; Chukwuka and Ojo, 2014).

Miranda et al. (2015) isolated 14 compounds from ethyl acetate extracts of the leaves, stems, and roots of *T. diversifolia* and 2 previously unreported sesquiterpene lactones; 8β-*O*-(2-methylbutyroyl) tirotundin and 8β-*O*-(isovaleroyl) tirotundin in the inseparable mixture. Their structures were determined by spectroscopic analysis, including NMR techniques and mass spectrometry (Miranda et al., 2015). Sesquiterpenes, diterpenes, monoterpenes, and alicyclic compounds isolated from the leaves, stem, and flowers have shown biological activities against insect pests (Obafemi et al., 2006).

The insect feeding deterrent characteristics of this plant have been linked to presence of 6-methoxyapigenin, tagitinins (A, B,C and F), diversiform, tirotundin, tithonine and sulphurein (Challand and Willcox, 2009). Tagitinins A, C, and hispidulin possessed insect antifeedant potential when evaluated against fourth instar caterpillar eri silkworm (*Philosamia ricini* Hutt.) (Dutta et al., 1986). The acaricidal and oviposition inhibitory effects of *T. diversifolia* extracts on *T. urticae* could mainly be due to tagitinin C and A's major role as repellent and antifeedant agents against the two-spotted spider mite, *T. urticae* (Pavela et al., 2016). Sesquiterpene lactones have also shown antifeedant activities on several arthropod pests (Susurluk et al., 2007). Tagitinin C exhibited pronounced antifeedant activity on the caterpillar *C. lacinia* (Ambrósio et al., 2008). Phenolic acids and flavonoids

Table 1
Sesquiterpene lactones isolated from *T. diversifolia.*

Chemical Compound	Class	Reference
8β-*O*-(2-methylbutyroyl)tirotundin	Germacranolides	Miranda et al. (2015)
8β-*O*-(isovaleroyl)tirotundin	Germacranolides	Miranda et al. (2015)
3β-acetoxytithifolin	Germacranolides	Miranda et al. (2015)
3-methoxytirotundin	Germacranolides	Miranda et al. (2015)
3α-acetoxycostunolide	Germacranolides	Miranda et al. (2015)
Tirotundin (Tagitinin D)	Germacranolides	Herz and Sharma (1975)Baruah et al. (1979)
Tagitinin A	Germacranolides	Baruah et al. (1979)
Tagitinin C	Germacranolides	Baruah et al. (1979), Goffin et al. (2002)
Tagitinin G	Germacranolides	Zhao et al. (2012)
tagitinin H	Germacranolides	Zhao et al. (2012)
tagitinin I	Germacranolides	Zhao et al. (2012)
1β-methoxydiversifolin 3-O-methyl ether	Germacranolides	Gu et al. (2002), Kuroda et al. (2007)
3α-(acetoxy)diversifolol	Eudesmanolide	Kuo and Chen (1998)
methyl 3α-acetoxy − 4α-hydroxy − 11(13)-eudesmen − 12-oate	Eudesmanolide	Kuo and Chen (1998)
Diversifolol	Eudesmanolide	Kuo and Chen (1998)
Tithofolinolide	Eudesmanolide	Gu et al. (2002)
3β-acetoxy-8β-isobutyryloxyreynosin	Eudesmanolide	Gu et al. (2002)
8β-(Isobutyroyloxy)-4-oxo-3,4-secoguai-11(13)-ene-12,6α;3,10α-diolide	Guaianolides	Ambrósio et al. (2008)
4α,10α-dihydroxy-3-oxo-8β-isobutyryloxyguai-11(13)-en-6α,12-olide	Guaianolides	Ambrósio et al. (2008)
3-hydroxy-8β-(isobutyroyloxy)leucodin-11(13)-ene	Guaianolides	Ambrósio et al. (2008)
8β-isobutyryloxycumambranolide	Guaianolides	Kuo and Chen (1998)
Hispidulin		Kuroda et al. (2007), Pereira et al. (1997)
2α-hydroxytirotundin	Germacranolides	Gu et al. (2002)
1β,2α-epoxytagitinin C	Germacranolides	Gu et al. (2002)
1-acetyltagitinin A	Germacranolides	Kuo and Chen (1998)
1α-hydroxytirotundin 3-O-methyl ether	Germacranolides	Kuroda et al. (2007)
Tagitinin E	Germacranolides	Schuster et al. (1992)
Tagitinin F	Germacranolides	Zdero et al. (1987)
Tagitinin F 3-O-methyl ether	Germacranolides	Zhao et al. (2012)
Tagitinin C methyl butyrate	Germacranolides	Ambrósio et al. (2008)
Tirotundin 3-O-methyl ether	Germacranolide	Ambrósio et al. (2008)
1 β,2α -epoxy tagitinin C	Germacranolides	Zdero et al. (1987)
1β-methoxydiversifolin	Germacranolides	Kuroda et al. (2007)
1α-hydroxydiversifolin 3-O-methyl ether	Germacranolides	Pereira et al. (1997) Kuroda et al., 2007);
2-O-methyl derivative of tagitinin B	Germacranolides	Pereira et al. (1997) Kuroda et al. (2007);
Acetyltagitinin E	Germacranolides	Wu et al. (2001)
1,3-dihydroxy − 3,10-epoxy − 8(−2-methylpropanoyloxy)-germacra − 11(13)-ene − 6,12-olide	Furanoheliangolides	Herrera et al. (2007)
1,3-dihydroxy − 3,10-epoxy − 8-(2-methylpropanoyloxy)-germacra − 4,11(13)-diene − 6,12-olide	Furanoheliangolides	Herrera et al. (2007)
1,3-dimethoxy − 3,10-epoxy − 8-(2-methylpropanoyloxy)-germacra − 4,11(13)-diene − 6,12-olide	Furanoheliangolides	Herrera et al. (2007)
1-hydroxy − 3-methoxy − 3,10-epoxy − 8-(2 methylpropanoyloxy)-germacra − 4,11(13)-diene − 6,12-olide	Furanoheliangolides	Herrera et al. (2007)

are believed to be responsible for reduction in the arthropod's growth and reproduction (Todd et al., 1971; Arancon et al., 2007). Caffeoylquinic acids such as chlorogenic acid – marker compounds from *T. diversifolia* methanolic extract, is capable of reducing the growth of the cabbage looper, *Trichoplusia ni* Hubner, when incorporated into artificial diet (Beninger et al., 2004). Tagitinins A, C, and hispidulin showed insect antifeedant potential when evaluated against fourth instar caterpillars eri-silk worm, *P. ricini* (Dutta et al., 1986). In a study conducted by Green et al. (2017), one of the active fractions possessing taginin A from *T. diversifolia* methanol extract was toxic to cowpea beetle, *Callosobruchus maculatus* (Fabricius, 1775)(Coleoptera: Bruchidae). Therefore the isolated tagitinin A identified using H^1 and C^{13}-NMR was responsible for the insecticidal activity of *T. diversifolia* against the cowpea beetle (Green et al., 2017). The mode of action of sesquiterpene lactones (Fig. 3) is not yet confirmed though Schmidt (2006) postulated that may be mediated by general chemical mechanisms like alkylation of nucleophiles through their α, β- or α, β, γ-unsaturated carbonyl structures (e.g.α-methylene-γ-lactones) or by receptor-mediated interactions. It is the presence of the γ-lactone group that closes towards C-6 and C-8; very often having an exocyclic α-methylene group (i.e. α-methylene-γ-lactones) that forms the basis of the biological activity of these groups. In principle, the possession of α-methylene-γ-lactones and incorporation of an epoxide, hydroxyl, chlorohydrin, unsaturated ketone or O-acyl group adjacent to the α-CH_2 of γ-lactone enhances the reactivity of the conjugated lactone towards biological nucleophiles (Rodriguez et al., 1976).

From the review, the chemical basis of most pesticidal activity of *T. diversifolia* crude extracts against many pests has not been reported including toxic principles and toxic components. Furthermore, it is necessary to find out if multiple components in plant extracts are responsible for insects' behavioral changes on exposure to this plants' extract. A study that evaluates the chemical parameters, phytochemical value and pesticidal activity of different crude extracts (for example, decoctions, leaf infusion, etc.) of *T. diversifolia* would be important to fingerprint this plant as one of the natural sources of traditional pesticides that guarantees positive healthy remedies. Different bioactive makers with positive health properties would be obtained and the total phytochemical content thus evaluated.

3.2.2. Phytochemical studies and isolated bioactive compounds of T. vogelii

Huan-Huan et al. (2009) isolated two compounds: a sesquiterpene; (1β,6α,10α)-guai-4(15)-ene-6,7,10-triol, and a lignin; (+)-lariciresinol 9^1-stearate from aerial parts of *T. vogelii.* This was in addition to the ones which were earlier isolated i.e. two sesquiterpenes: teclenone B, (1β,7R*)-opposit-4(15)-ene-1,7-diol and pinoresinol, a lignan. In another study, flavonoid glycosides and flavonoid aglycones

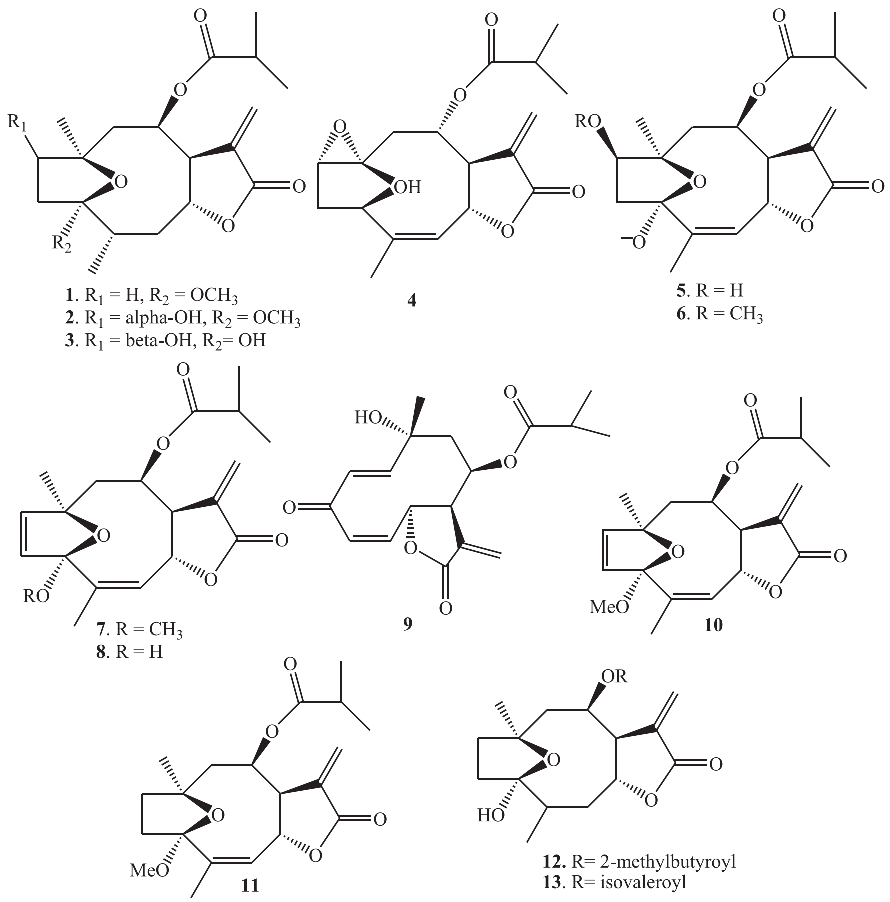

Fig. 3. 1–13 some sesquiterpene lactones and bioactive compounds found in *T. diversifolia*: (Pal et al., 1976; Baruah et al., 1979; Goffin et al., 2002; Kuroda et al., 2007; Gu et al., 2002; Ambrósio et al., 2008; Zhao et al., 2012; De Toledo et al., 2014; Miranda et al., 2015; Green et al., 2017). Tirotundin-3-***O***-methyl ether (**1**), 1*β*-hydroxytirotundin-3-***O***-methyl ether (**2**), tagitinin A (**3**), deacetylvguiestin (**4**), 1*β*-hydroxydiversifolin-3-***O***-methyl ether (**5**), 1*β*-hydroxytirotundin-1,3-***O***-dimethyl ether (**6**), tagitinin F-3-***O***-methyl ether (**7**), tagitinin F (**8**), tagitinin C (**9**), tagitinin F-3-*O*-methyl ether (**10**), 3-methoxytirotundin (**11**), 8*β*- ***O***-(2-methylbutyroyl)- tirotundin (**12**), 8*β*- ***O***-(isovaleroyl)- tirotundin (**13**).

were reported by Stevenson et al. (2012) from methanol extracts of *T. vogelii* using LC–UV–MS/MS methods. In this study, the quercetin 3-O-galactoside, quercetin 3-O-glucoside, and a quercetin 3-O-pentoside were the most abundant flavonoid glycosides (Stevenson et al., 2012). Among the flavonoid aglycones, rotenoids: deguelin and tephrosin were identified as major components with sarcolobine, rotenone, and α-toxicarol being minor (Ingham, 1983; Marston et al., 1984; Stevenson et al., 2012). Stevenson et al. (2012) further isolated the known prenylated flavanone compounds: obovatin 5-methyl ether and *Z*-tephrostachin and the previously unknown flavonoid aglycones (Table 2) by semi-preparative HPLC. Table 2 shows some of the phytocompounds which have been isolated and identified from *T. vogelii*. The isolation study of flavonoid aglycones by Stevenson et al. (2012) revealed two chemical varieties of *T. vogelii*: one containing rotenoids as the main type of flavonoid aglycones (chemotype 1) and the other containing flavanones as the main aglycone type (chemotype 2). The rotenoids in chemotype1 were deguelin, rotenone, sarcolobine, tephrosin, and α-toxicarol (Fig. 4). They have in common a chromanochromanone (4-ring structure) and are reported to be highest in the leaves (Irvine and Freyre, 1959; Kalume et al., 2012). Chemotype2 contain no rotenoids, but only prenylated flavanones such as obovatin 5-methylether, flavones, and flavanols (Stevenson et al., 2012). The difference between the chemical structures of the two chemotypes is that the oxygenated cycles in chemotype 1 are cis-fused unlike in chemotype 2 (Fig. 5). The dihydrofuran ring in the former chemotype is believed to possess the maximal biological activity (pesticidal activity) (Bruneton, 2008).

According to earlier literature (e.g. Matsumura, 1985), the bioactive component for all the insecticidal effects of *T. vogelii,* is rotenone. There had been no published work to corroborate 'gray' literature citation that rotenoids in the leaves of *Tephrosia* spp. to be insecticidal against stored product pests, until 2012 (Stevenson et al., 2014). Belmain et al. (2012) showed categorically that rotenoids are indeed the biologically active compounds to bruchids. The rotenoids: deguelin, rotenone, sarcolobine, tephrosin, and α-toxicarol, thus chemotype 1 are required for pest control efficacy while chemotype 2 is non-pesticidal (Belmain et al., 2012). The authors (Belmain et al., 2012) further added that rotenone and deguelin are the major rotenoid compounds in *T. vogelii*. Belmain et al. (2012) further reported that rotenone itself plays only a relatively minor part in pesticidal effect compared to the much more abundant compound deguelin. Deguelin is the most effective on adult bruchids when 2% leaf extract of *T. vogelii* was coated on the grain of cowpeas (Stevenson et al., 2010). The two components (deguelin and tephrosin) had toxic principles and may possess oviposition potential (Belmain et al., 2012). Tephrosin and deguelin are structurally related, but the former was much less active at 100 ppm than the later although the molecular structures differ by one additional hydroxylation (Belmain et al., 2012). This led to the conclusion that not all rotenoids have similar activities at the same concentration and the source of *T. vogelii* could be important, so the plant material being used by farmers need to be higher in deguelin (Stevenson et al., 2010; Belmain et al., 2012). Two of the other rotenoids identified in *T. vogelii* (sarcolobine and toxicarol) had similar biological activities to deguelin (Belmain et al., 2012). Njiru (2006) suggested that a combination of rotenone, one of the major

Table 2
Flavonoids and other compounds isolated from *T. vogelii.*

Chemical compound	Phytochemical class	Author
(1β,6α,10α)-guai-4(15)-ene-6,7,10-triol	Sesquiterpene	Huan-Huan et al. (2009)
(+)-lariciresinol 9[1]-stearate	Lignan	Huan-Huan et al. (2009)
Teclenone B	Sesquiterpene	Al-Rehaily et al. (2002), Huan-Huan et al. (2009)
(1β,7R*)-opposit-4(15)-ene-1,7-diol	Sesquiterpene	Iijima et al. (2003) Huan-Huan et al. (2009)
Pinoresinol	Lignan	Casabuono and Pomillo (1994); Huan-Huan et al. (2009)
Quercetin 3-O-robinobioside	Flavonoid glycosides	Stevenson et al. (2012)
Quercetin 3-O-rutinoside	Flavonoid glycosides	Stevenson et al. (2012)
Quercetin 3-O-galactoside	Flavonoid glycosides	Stevenson et al. (2012)
Quercetin 3-O-glucoside	Flavonoid glycosides	Stevenson et al. (2012)
Isorhamnetin 3-O-galactoside	Flavonoid glycosides	Stevenson et al. (2012)
Isorhamnetin 3-O-glucoside	Flavonoid glycosides	Stevenson et al. (2012)
Isorhamnetin 3-O-pentoside	Flavonoid glycosides	Stevenson et al. (2012)
Deguelin	Flavonoid aglycones (rotenoid)	Dagne et al. (1989), Ye et al. (2008)
Tephrosin	Flavonoid aglycones (rotenoid)	Dagne et al. (1989), Ye et al. (2008)
Sarcolobine	Flavonoid aglycones (rotenoid)	Andrei et al. (1997)
Rotenone	Flavonoid aglycones (rotenoid)	Blaskó et al. (1989)
α-Toxicarol	Flavonoid aglycones (rotenoid)	Wangensteen et al. (2005)
(2**S**)-5-methoxy-6″,6″-dimethylpyrano[2″,3″:7,8]flavanone	Flavonoid aglycones (flavanone)	Stevenson et al. (2012)
5,7-dimethoxy-8-(3-hydroxy-3-methylbut-1*Z*-enyl)flavone (*Z*-tephrostachin)	Flavonoid aglycones (flavanone)	Stevenson et al. (2012)
(2*S*)-5,7-dimethoxy-8-(3-hydroxy-3-methylbut-1*Z*-enyl)flavanone	Flavonoid aglycones (flavanone)	Stevenson et al. (2012)
(2**S**)-5,7-dimethoxy-8-(3 methylbut-1,3-dienyl)flavanone	Flavonoid aglycones (flavanone)	Stevenson et al. (2012)
(2**S**)-4′-hydroxy-5-methoxy-6″,6″ dimethylpyrano[2″,3″:7,8]flavanone	Flavonoid aglycones (flavanone)	Stevenson et al. (2012)
(2**S**)-5-methoxy-6″,6″-dimethyl-4″,5″ dihydrocyclopropa[4″,5″]furano[2″,3″:7,8] flavanone	Flavonoid aglycones (flavanone)	Stevenson et al. (2012)
(2**S**)-7-hydroxy-5-methoxy-8-prenylflavanone	Flavonoid aglycones (flavanone)	Stevenson et al. (2012)
(2*R*,3*R*)-3-hydroxy-5-methoxy-6″,6″-dimethylpyrano[2″,3″:7,8]flavanone	Flavonoid aglycones (flavanone)	Stevenson et al. (2012)

ingredients of *T. vogelii* with pyrethrins is a safe insecticidal formulation to humans and the environment. The biological activities of the huge amount of nonrotenoid compounds/fractions that go to waste as undesirable substances after rotenoids have been removed for pesticides are unknown. Thus chemotype 2 of *T. vogelii* is not utilized. Perhaps they possess synergistic effect, which could be investigated. Since it is reported that a phytocomplex (active principles and other plant components) can contribute to the overall biological effect (Donno et al., 2015). From this information, the chemical basis of the pesticidal activity of *T. vogelii* when used as a single botanical pesticide is quite well known although there is need to look at the *T. vogelii* chemotypes available to farmers in other regions due to chemical variability and the potential implications this might have on its pesticidal use and advise accordingly. In addition, the rotenoid content determination and stability of *T. vogelii* rotenoid compounds need to be addressed. Furthermore, investigations on joint effects of both *T. diversifolia* and *T. vogelii* extracts against many pests is still not reported and thus the dosages of compounds responsible for the effects. It is also not yet confirmed whether the chemistry of the pesticidal activity of the essential oil from *T. vogelii* could indicate the existence of different chemotypes. This is because some plants have shown morphologically distinct varieties of essential oils when grown in different locations subsequently affect their insecticidal potentials such as *Lippia javanica* (Burm. f.) (Kamanula et al., 2017).

3.3. Characterization of crude extracts for bioactive compounds and health-related aspect of biomarkers as reported in other researches

Plant-based compounds when exploited sustainably could become alternative sources of income, as well as an affordable and accessible natural health remedy. This would require the characterization of their crude extracts which provides new information on their ethnobotanical uses. Complete characterization of an extract is possible for commonly used and well-researched botanical plants, but this is not easy for more complex and less studied plants where standards are not readily available or components have not been well characterized (Wheat, 2013). Even the most sensitive and advanced method of metabolite profiling results in a long list of compounds identified from extracts. Several analytical techniques have been used in the study of crude extracts in order to characterize plant species as sources of biologically active compounds. These include classic bioassay-guided isolation and two approaches based on analysis of whole extract using either NMR or LC–MS spectroscopic techniques (Wheat, 2013).

The classic bioassay-guided fractionation involves targeted isolation of bioactive compound (Pieters and Vlietnik, 2005). This involves distinct steps including preliminary extraction, drying, dissolving, precipitating and filtering followed by multiple chromatographic steps to separate individual component. Along the way, the activity of the separated fractions is evaluated to get rid of the non-active ones and finally arrive at an active fraction (Seidel and Taylor, 2004). Large amount (kilograms) of starting materials are used to identify a single compound (Kumar et al., 2012) and more often than not it is a known compound (Othman et al., 2006; Freitas et al., 2009; Schwaiger et al., 2011), casting doubt on why use it for isolation. In addition, Classic bioassay-guided isolation, in rare instances results in new compounds being identified (Ferchichi et al., 2012).

Whole extract analysis using LC–MS and tandem LC–MS/MS spectrometry is a recently developed method for determining the constituent compound in a crude botanical extract. The method in principle requires very little sample materials and can detect thousands of metabolites relatively quickly with higher accuracy (Steinmann and Ganzera, 2011; Wheat, 2013). The method involves first an efficient HPLC fractionation of crude extract with the higher pressures and control of solvent composition significantly reducing running time and optimizing separations. Reverse phase chromatography using nonpolar solid phase often C18 and a polar liquid phase is capable of separating polar and semi-polar compounds generally found in botanical extracts. Effluents from the chromatographic column are directly fed into a mass spectrometer where various ionization methods can be utilized, preferably electron spray ionization (ESI). ESI is preferable because ionization forms intact molecular ions that enable identification of unknown compounds (Bowen and Northen, 2010; Xiao et al., 2012) and when performed at high resolution, the molecular formula can be deduced with high certainty from the accurate mass. An alternative approach and

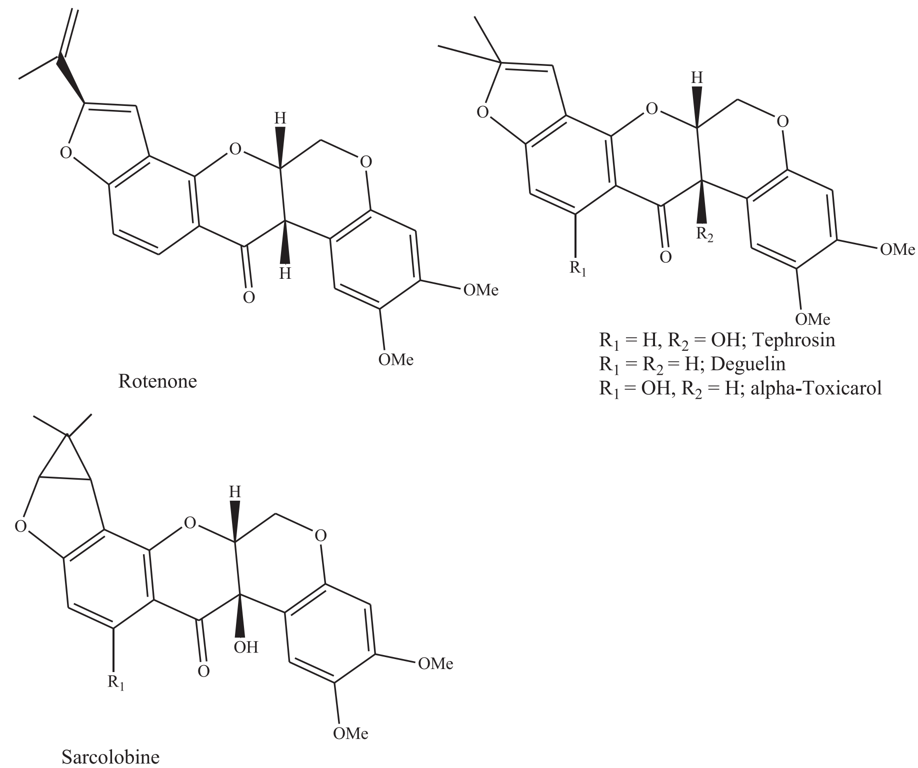

Fig. 4. Structures of rotenoids in *T. vogelii* (Belmain et al., 2012).

perhaps complementarily used to detect a constituent in a crude extract is the use of high field NMR spectroscopy (Wheat, 2013). Although high field and resolution with the various application of 1D and 2D experiments, NMR can, however, provide a useful 'fingerprint' on a range of compounds present in crude extract (Ward et al., 2003).

The study of phytochemical fingerprinting for all biomarkers of plants can provide information about the health properties of biomolecules which would guarantee a natural health remedy for humans after biological applications (Donno et al., 2017; Rakotoniaina et al., 2018). Studies have been made on relative health-promoting properties of phytochemicals and the influence of agro-environmental conditions on their concentration together with extraction and analytical methods used to identify the same phytochemicals in other researches. Donno et al. (2017) studied the phytochemicals from *Brachylaena ramiflora* (DC.) Humbert leaves infusions and bark decoctions using high-performance liquid chromatography-diode array detector (HPLC-DAD) for their antioxidant activity. Sixteen and twenty-three biomarkers possessing health properties to humans were, respectively, identified from the plant's leaf infusions (major compounds: quinic acid, chlorogenic acid, and g-terpinene) and bark decoctions (castalagin, citric acid, and chlorogenic acid) (Donno et al., 2017). This study fingerprinted this plant as a natural source of antioxidant. The study reported that the highest polyphenol content was found in the leaves leaf decoctions than infusions but the reverse was true for terpenic compounds in *B. ramiflora* i.e. infusions in contrast had higher terpenic compounds. In a related study, Rakotoniaina et al. (2018) determined the phenolic and organic compounds in the leaves and stems of *Chrysophyllum boivinianum* (Pierre) Baehniin in relation to their biological activities and local uses by spectrophotometric and chromatographic analysis. Leaf extracts contained the highest amount of total polyphenolic compounds (TPC) (805.16 ± 1.08 mgGAE/100 gDW), followed by leaf infusions at (477.87 ± 38.49 mgGAE/100 gDW). The stem extracts with TPC (249.12 ± 7.11 mgGAE/100 gDW) and stem decoctions (191.66 ± 14.88 mgGAE/100 gDW) had lower TPC than did the leaf extracts. Leaf infusions showed much higher antioxidant activity than the leaf extracts chlorogenic acid, caffeic acid and gallic acid (Rakotoniaina et al., 2018). The authors showed that the leaves of *C. boivinianum* showed a higher content of bioactive compounds than

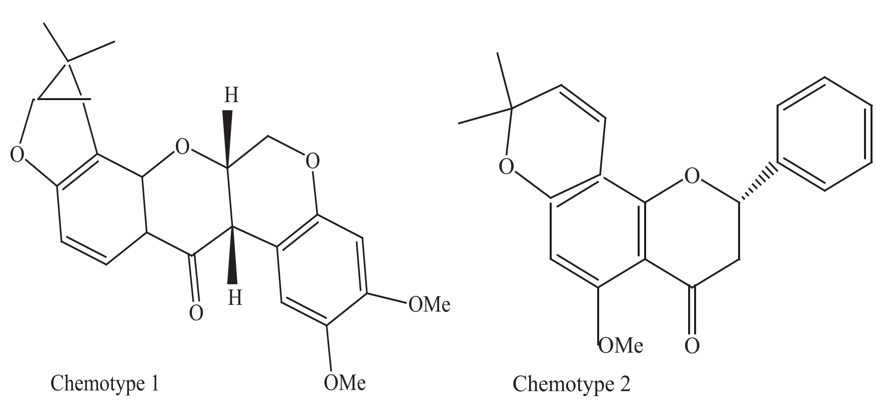

Fig. 5. Structures of chemotype1 and chemotype2 of *T. vogelii* (Stevenson et al., 2012).

the stems and the infusions gave the best method for extracting biomolecules high in health properties (Rakotoniaina et al., 2018). The influence of agro-environmental conditions affects the concentration of the same phytochemicals identified in other researches. For example, different locations thus different environment conditions lead to variation in the bio-markers (total phenol and anthocyanin contents) possessing health benefits from strawberry cultivars analyzed using liquid chromatography-mass spectrometric (LC–MS) technique (Josuttis et al., 2013). Similar studies have not been reported for *T. diversifolia* and *T. vogelii* since the need of assuring the safety and efficacy of their extracts, bioactive compounds and their break down metabolites increases as well.

4. Conclusion

This review critically summarizes the current evidence on the traditional pesticidal use, phytochemistry of *T. diversifolia* and *T. vogelii* and the health related aspects of bio-markers as reported in other researches which would trigger further research on these plants with respect to their pesticidal applications. Most of the pesticidal applications of *T. diversifolia*, published in the last 10 years show that its crude extracts have been used rather than pure isolated compounds for pesticidal activities. The mechanisms of action of these extracts are not fully demonstrated thus the gap on its pesticidal activity. We have discussed the extent of chemical variation of pesticidal activity of *T. vogelii* leaf materials as reported in previous literature. We also raised a question on whether there could be distinct chemotypes based on different components of the volatile oils. This variation implies that preliminary tests on *T. vogelii* material is needed to find out whether it is pesticidal or not before applying the plant extract on crops. Furthermore, two plants discussed here could give a stronger joint effect against insect pests than individual plant extracts. The challenges of isolating and characterizing complex crude extracts that comprise a multitude of compounds are solved by hyphenating high-performance liquid chromatography and mass spectrometry (LC–MS). The LC–MS provides the user with a multitude of technical options and applications.

Competing interests statement

None.

Contributors

All authors contributed equally to this research.

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